STUDIES ON TREMATODES OF WOODS HOLE: THE LIFE CYCLE OF LEPOCREADIUM SETIFEROIDES (MILLER AND NORTHUP), ALLOCREADIIDAE, AND THE DESCRIPTION OF CERCARIA CUMINGIAE N. SP.[1]

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The results reported in this paper were obtained during the summers of 1936 and 1937 at the Marine Biological Laboratory at Woods Hole, Mass. They include a possible life cycle of *Lepocreadium setiferoides* (Miller and Northup, 1926) and the description of a new species of trematode, *Cercaria cumingiae,* with observations on its life cycle. In the adult stage, *Lepocreadium setiferoides* parasitizes the flounder and hence may be of some economic importance.

Material and Methods

The various hosts of the trematodes described in this paper were collected near Woods Hole. The mollusk, *Nassa obsoleta,* the first intermediate host of *L. setiferoides,* was secured at Quisset Harbor. The second intermediate hosts of this trematode, the turbellarian, *Procerodes warreni,* and the annelid, *Spio* sp., were collected at Nobska Beach and Sheep Pen Harbor. The flounders and sand dabs, that serve as hosts of the adult worms, were collected from various shallow waters. *Cumingia tellinoides,* which serves as both the first and second intermediate hosts of *Cercaria cumingiae,* was collected from a number of localities.

The mollusks were isolated in finger bowls and examined under the dissecting microscope for the emergence of cercariae. When an infected *Nassa* was found, the second intermediate hosts were placed with it in a finger bowl of sea water. After an interval of several days, the infected second intermediate hosts were fed to flounders and sand dabs. *Nereis* was used as a food supply for the fishes since it was found that this worm would not serve as a second intermediate host of *L. setiferoides.*

[1] This work was done under the direction of Dr. R. M. Cable to whom my thanks are hereby expressed.

Neutral red was used in the study of living material, particularly to stain glandular tissues. Living material was flattened under light cover-glass pressure and fixed in a saturated aqueous solution of mercuric chloride. Mayer's paracarmine was used to stain toto-mounts.

Observations

The life cycle of *Lepocreadium setiferoides* includes the development of the larval stages in *Nassa obsoleta,* the encystment of the metacercaria in the turbellarian, *Procerodes warreni,* and the annelid, *Spio* sp. and the development of the adult in the sand dab and flounder.

The Redia (Fig. 2)

The rediae develop in the digestive gland of *Nassa obsoleta.* They are elongate, with bluntly rounded anterior end and pointed posterior end. A collar and ambulatory processes are lacking. No birth pore was observed. In flattened, fixed specimens, the rediae vary in length from 0.43 mm. to 0.94 mm.; in width from 0.088 mm. to 0.11 mm. The pharynx measures approximately 0.037 mm. in length by 0.040 mm. in width. Young rediae contain as many as thirty germ balls but no mature cercariae. A sac-shaped gut extends posteriorly from the pharynx as far as one-third the length of the redia.

The Cercaria (Fig. 3)

The cercariae possess two conspicuous eye spots near the level of the pharynx. The tail is provided with lateral tufts of setae.

Observations and measurements of living cercariae under light cover-glass pressure are as follows: the body is 0.305 mm. long by 0.10 mm. in maximum width at the level of the ventral sucker; the tail is 0.576 mm. long by 0.047 mm. wide at its proximal end; the caudal setae are 0.073 mm. long at the proximal end of the tail and somewhat shorter distally. The setae are arranged in transverse rows, of from four to seven setae each, on either side of the tail. Miller and Northup (1926) stated that there were about thirty pairs of setae groups on the tail, but in the writer's specimens, thirty-five pairs of tufts were found in addition to an unpaired group of setae at the tip of the tail. The bases of the setae are expanded and fused with one another. The tail is composed of an outer sheath of muscles and an inner vacuolated, parenchymatous core. The cuticula of the body is provided with small spines which decrease in number towards the posterior end of the body. The body varies in shape with the degree of contraction. The oral sucker is about 0.058 mm., the ventral sucker

about 0.044 mm. in diameter. The two conspicuous, black eye spots are about 0.015 mm. in diameter and are located approximately midway between the two suckers. The prepharynx varies in length with the degree of contraction of the body, generally being very short. The pharynx measures 0.022 mm. in length by 0.015 mm. in width. The esophagus is approximately as long as the pharynx. The intestine is rudimentary, sometimes appearing as two strings of cells, staining deeply with neutral red, extending from the esophagus to near the posterior end of the body. Eight cephalic glands are present on each side of the body and partially surround the ventral sucker. Their ducts pass anteriorly and open to the exterior at the anterior end of the body. These glands stain deeply with neutral red but in the same general region there appear to be other glands that do not absorb this stain. From ten to fifteen cystogenous glands are scattered irregularly through each half of the body.

The reproductive system is well developed. The two testes have slightly lobed or smooth margins and are located in the posterior one-third of the body. The ovary lies slightly to the right of the mid-line between the anterior testis and the ventral sucker. The accessory reproductive structures are not readily distinguishable.

The excretory bladder is a conspicuous, tubular sac which extends from the posterior end of the body to a short distance in front of the ventral sucker. It contains numerous, refractile, discoid concretions whose lines of accretion may be clearly seen under the oil immersion lens. At the posterior end of the bladder there is a muscular sphincter about 0.006 mm. long by 0.005 mm. wide. The excretory pore opens dorsoventrally. The ciliated, main collecting tubes arise from the excretory bladder at the level of the testes and pass cephalad as far as the level of the anterior margin of the ventral sucker before each divides into an anterior and a posterior secondary collecting tube which supply their respective regions of the body. The secondary tubes divide into capillaries which end in flame cells. There are 24 flame cells on each side of the body which appear to be arranged in groups of threes. However, one of the capillaries of each group joins the secondary collecting tube independently of the other two.

The cercariae emerge both diurnally and nocturnally and are photonegative. They swim rapidly about by lashing the tail.

The Metacercaria

The cercariae were observed to penetrate and encyst in the triclad turbellarian, *Procerodes warreni,* and in spionid worms. The tail is shed in the process of penetration. These experimental hosts may not

be the natural ones, particularly *Procerodes* which is found in rocky regions which are not the habitats of the flatfish. The metacercaria does not increase much in size but the reproductive systems become more mature.

The Adult (Fig. 1)

Natural infections of *Lepocreadium setiferoides* were found in most of the small flounders and sand dabs so that suitable experimental animals, known to be uninfected with this parasite, were not available. However, the flounders were fed infected *Procerodes* and spionids over a period of about one month and various stages of development from the metacercarial to the adult were obtained. The following description and measurements are based upon observations of 35 preserved worms. The cuticula is covered with scale-like spines about 0.005 mm. long at the anterior part of the body but somewhat smaller at the posterior end. The body length varies from 0.35 mm. to 0.58 mm. with an average of

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EXPLANATION OF PLATES

All drawings except Fig. 7 were made with the camera lucida.

Abbreviations used:

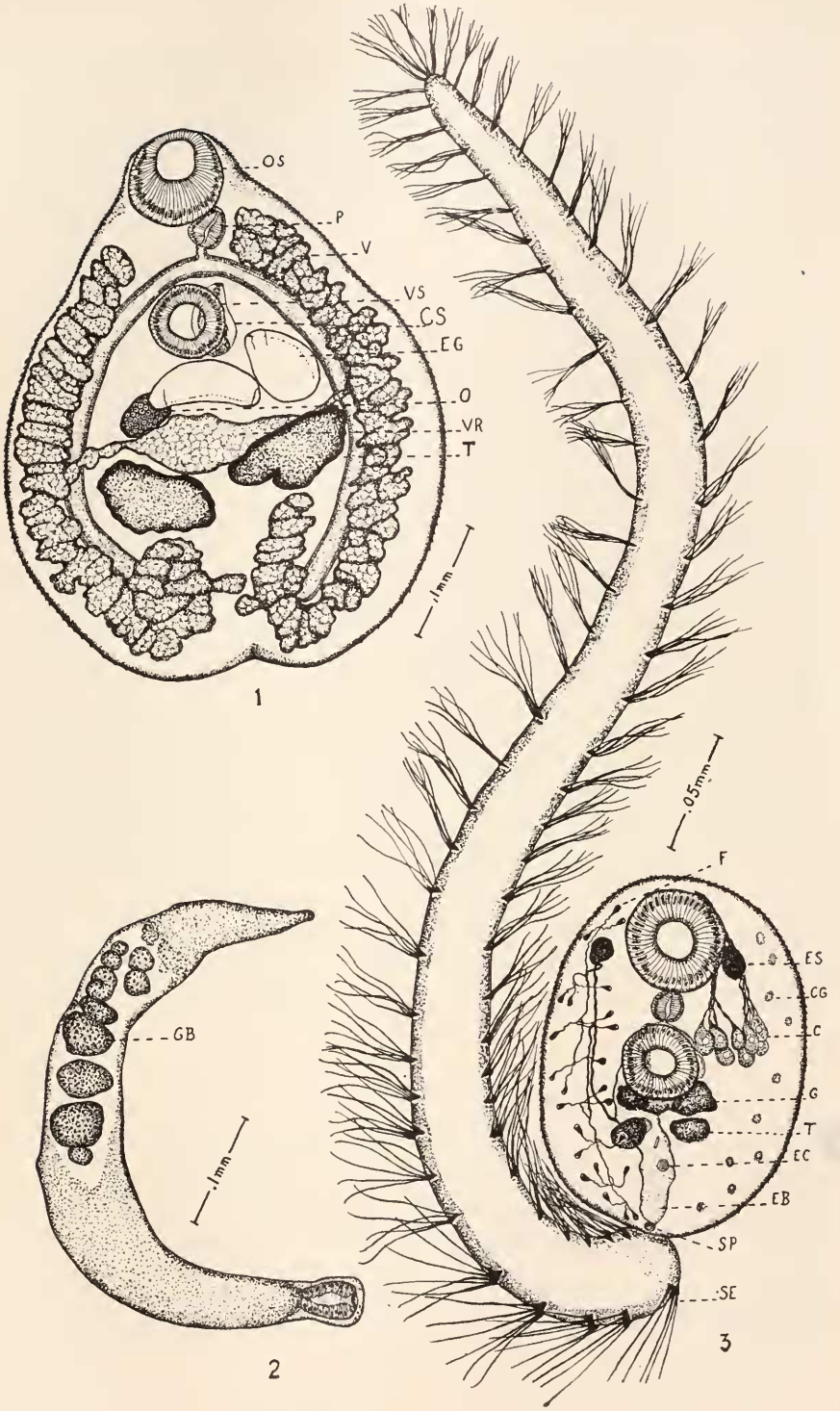
C cephalic gland
CG cystogenous gland
CS cirrus sac
E esophagus
EB excretory bladder
EC excretory concretion
EG egg
ES eyespot
F flame cell
G genital primordium
GB germ ball
I intestine
L lappet
O ovary
OS oral sucker
P pharynx
PP prepharynx
SE seta
SP sphincter
T testis
V vitellaria
VR vitelline reservoir
VS ventral sucker

Description of Plate I

All figures concern *Lepocreadium setiferoides.*

Fig. 1. Ventral view of adult.
Fig. 2. Young redia showing germ balls.
Fig. 3. Ventral view of cercaria.

PLATE I



0.43 mm. The body width varies between 0.19 mm. and 0.35 mm., averaging 0.26 mm. The oral sucker is approximately circular and averages about 0.077 mm. in diameter. The mouth opens into a very short prepharynx. The pharynx averages 0.045 mm. in length by 0.043 mm. in width. The intestine bifurcates in front of the ventral sucker and the intestinal ceca extend to near the posterior end of the body.

The vitellaria extend from the anterior margin or the middle of the pharynx to the posterior end of the body where they may become confluent. Vitelline ducts carry the yolk material to a reservoir located near the ovary. The ovary is located to the right of the mid-line of the body between the anterior testis and the ventral sucker. It is oval in shape and averages about 0.04 mm. by 0.044 mm. A short Laurer's canal opens to the exterior on the dorsal surface of the body. The seminal receptacle is spherical in shape and approximates the size of the ovary although its size varies considerably. The uterus coils between the anterior testis and the ventral sucker to open at the genital pore to the left of the anterior margin of the ventral sucker. The eggs are few in number and very large in proportion to the size of the worm. Their length varies from 0.05 mm. to 0.11 mm., averaging 0.066 mm.; their width varies from 0.04 mm. to 0.073 mm., with an average of 0.07 mm.

The two testes are obliquely arranged in the posterior half of the body. They are oval in outline with smooth or lobed margins. The right testis averages 0.066 mm. by 0.076 mm. while the left testis averages 0.07 mm. by 0.074 mm. The cirrus sac is oval in outline and extends from the genital pore to a point slightly posterior to the ventral sucker. The cirrus is tubular and slightly coiled. The prostatic cells are few in number. A seminal vesicle is located in the posterior part of the cirrus sac and in a few worms slight dilatations of the ductus deferens were observed external to the cirrus sac.

Discussion

Miller and Northup (1926) described the cercarial stage of this trematode as *Cercaria setiferoides*, stating that it was similar to *C. setifera* Müller, 1850 (described by Monticelli, 1914, and redescribed by Odhner, 1914). Palombi (1932, 1934*b*) has shown that Monticelli described several setiferous-tailed cercariae under the name, *Cercaria setifera*. A number of workers, including Villot (1875, 1879), Fewkes (1882), Huet (1891), Giard (1897), Odhner (1911), Dollfus (1925), and recently, St. Markowski (1936) and Hopkins (1937), have described setiferous-tailed cercariae. Some of these larvae have Y-shaped

excretory bladders and probably belong to the genus *Bacciger* as shown by Palombi (1934*a*). The setiferous-tailed cercariae described by Hopkins develop into adults belonging to the genera *Anallocreadium* and *Microcreadium*. It thus appears that the presence of setae on the tail, like many other characteristics of cercariae, is merely a larval adaptation with little phylogenetic significance. Palombi (1934*b*) has shown that one of the setiferous-tailed cercariae described by Monticelli develops into the adult trematode, *Lepocreadium album*. This parasite utilizes *Nassa mutabilis* and *Conus mediterraneus* as the first intermediate host, *Aplysea punctata, Tapes decussatus* and *T. aureus* as the second intermediate host, and *Blennius gattorugine* as the definitive host. All of these hosts differ from those in the life cycle of *L. setiferoides*. The adult *L. setiferoides* has not been previously described. It is most like *Lepocreadium ovalis* Manter, 1931, found in the intestine of the pinfish, *Lagodon rhomboides,* collected at Beaufort, N. C. The body of *L. setiferoides* is about half as large as that of *L. ovalis,* while the eggs are about the same size. Manter does not give the dimensions of the pharynx or a description of the excretory bladder, the latter organ being very conspicuous in *L. setiferoides*.

Lepocreadium setiferoides may be of some economic importance since it parasitizes a food fish. These parasites have been found in very small flounders and a young animal generally is more susceptible to the injurious effects of parasitism than an older animal. However, larger flounders, six to eight inches or more in length, rarely appear to be infected. This probably explains the fact that Linton did not find this species in his extensive study of fish parasites.

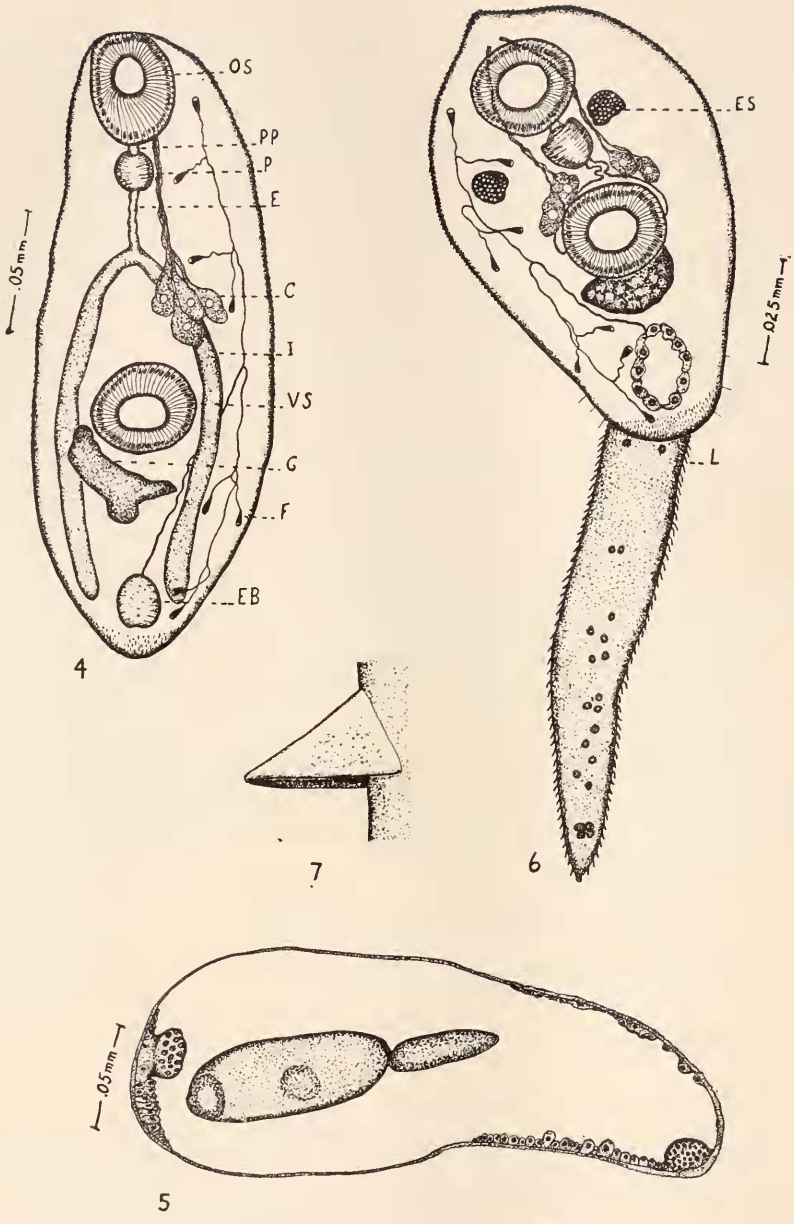
Cercaria Cumingiae N. Sp.

This trematode develops in sac-shaped sporocysts in the digestive gland of *Cumingia tellinoides*. Approximately 4 per cent of 175 *Cumingia* were found to be infected.

The Sporocyst (Fig. 5)

The sporocysts are simple, sac-shaped or tubular structures having at each end a concentration of cells that stain deeply with paracarmine. Otherwise, the walls of the sporocyst are very thin. Young sporocysts containing no germ balls or cercariae are approximately 0.20 mm. in length by 0.09 mm. in width. Older sporocysts containing 12 to 18 germ balls and cercariae are 0.58 mm. in length by 0.09 mm. in width, indicating that the growth of the sporocyst is principally in length.

PLATE II



DESCRIPTION OF PLATE II

All figures concern *Cercaria echinobigis* Cumingiae.

FIG. 4. Ventral view of metacercaria removed from cyst.
FIG. 5. Young sporocyst containing a cercaria.
FIG. 6. Ventral view of cercaria.
FIG. 7. Lappet from tail of cercaria (diagrammatic).

The Cercaria (Fig. 6)

Spination is limited to the cuticula of the anterior half of the body and a small area on the ventral surface near the posterior end. The body is oval in outline, varying in length and width with the degree of contraction. There are two conspicuous eye spots on the dorsal surface near the level of the pharynx. The oral sucker is subterminal and slightly smaller than the ventral sucker which is located in the mid-ventral region of the body. The ventral sucker bears about three rows of papillae. Immediately lateral and anterior to the ventral sucker there are four cephalic glands that stain deeply with neutral red. The ducts from these glands pass anteriorly to open externally on the anterior margin of the oral sucker.

The oral sucker is followed by a short prepharynx, a bulbous pharynx, a well-developed esophagus, and short intestinal crura which do not extend posterior to the ventral sucker. The short posterior extension of the intestinal branches is a rudimentary condition since they reach almost to the posterior end of the body in the metacercaria.

The excretory bladder is simple and sac-shaped and its wall is composed of a single layer of large granular cells. From the anterior portion of the bladder, the main collecting tubes pass laterally and anteriorly on each side of the body to the level of the ventral sucker where each divides into two secondary tubes, one of which extends anteriorly, the other posteriorly. Each secondary tube receives the capillaries of two pairs of flame cells so that the excretory formula equals $2[(2+2)+(2+2)]$.

The reproductive system is represented by an undifferentiated mass of cells located between the excretory bladder and the ventral sucker.

The tail of the cercaria is unusual in that it possesses, on each side, a row of cup-shaped lappets which are about 0.005 mm. long and can be raised to stand out at right angles to the tail or may be folded backward flat against the tail. There are fifty-two lappets on each side of the tail including the proximal first two which are not well developed. They may give the tail the appearance of being annulated.

Measurements in millimeters of cercariae killed and fixed under light cover-glass pressure are as follows: body length 0.1; width 0.06; oral sucker 0.026 by 0.027; ventral sucker 0.026 by 0.03; pharynx 0.01 long by 0.012 wide; eye spots 0.01 in diameter; tail, length 0.095 to 0.15, width 0.014 to 0.018. Measurements of living material give somewhat higher values.

In emerging from the host, *Cumingia tellinoides,* the cercariae are expelled through the excurrent siphon. They swim about in the sea water with the body folded upon itself and the tail describing figure-

eight movements pulling the body along. The larvae show a definite negative response to light. When one side of the finger bowl receives more light than the other, the cercariae swim directly to the region of lesser illumination. They are frequently carried back into the clam by the sucking action of the incurrent siphon. Upon coming in contact with the tissue of the siphons or foot of the clam, they quickly lose their tails, penetrate the tissue and encyst. The siphons may become so filled with cysts that portions of them are detached. These infected pieces of siphons may retain their ability to move, simulating the motion of annelids, and thereby attracting the attention of fishes. This detachment of portions of the siphons seems to be partially autotomous and partially due to the rupturing of the siphon tissue by the metacercariae. Occasionally, *Cumingia* were found to be naturally infected with this metacercaria but with a fewer number of cysts than in experimental infections.

The Metacercaria (*Fig. 4*)

The young metacercaria is similar to the cercaria in most respects but certain changes occur with an increase in age. These changes include: the loss of the tail when penetration takes place; the disintegration of the eye spots; the elongation of the intestinal ceca so that they extend to near the posterior end of the body; the increase in the number of cephalic glands to four on each side of the body; and some differentiation of the genital ducts. The excretory system is the same as in the cercaria except that the excretory bladder is relatively smaller in the metacercaria. Measurements in millimeters of metacercariae removed from their cysts and flattened under light cover-glass pressure are as follows: average body length 0.195; average body width 0.088; oral sucker 0.031 by 0.032; ventral sucker 0.029 by 0.032; pharynx 0.018 by 0.021.

Specimens of *Cumingia* were experimentally infected with metacercariae and then segregated for varying periods of time up to two weeks to allow the metacercariae to mature. These infected *Cumingia* were fed to specimens of *Fundulus* and *Paralichthys*. Upon subsequent examination, no worms were found in the *Fundulus* but from the intestine of one flounder over fifty small worms were recovered and definitely found to have developed from the metacercariae that were experimentally fed. Control fishes were negative for this species. The worms recovered had not developed sufficiently from the metacercarial stage to determine, with accuracy, the characteristics of the adult.

Discussion

Although *Fundulus* and *Paralichthys* did not serve as favorable definitive hosts, it is believed that some fish is involved in the life cycle of this trematode. This is suggested by the fact that *Cumingia* is a bottom-dwelling form, and since the siphons can be protruded some distance from the shell, they could be bitten off by fishes. In addition, the anatomical characteristics of this trematode suggest that it belongs to the family *Allocreadiidae,* the members of which, with few exceptions, are parasites of fishes.

The relationship of this species to other trematodes is uncertain since its life cycle is incompletely known. However, the arrangement of the principal excretory tubes, the sac-shaped excretory bladder, and the position of the genital primordium, suggest affinity to the family, *Allocreadiidae.* The cercaria has certain characters in common with *Cercaria myocerca* Villot, 1879, from *Scrobicularia tenuis.* Both of these larvae have the simple, sac-shaped type of excretory bladder. This seems rather significant since *C. myocerca* is a marine form with a setiferous tail and all other described species of setiferous-tailed marine cercariae have either a Y- or U-shaped, or a long tubular excretory bladder. Both species have eye spots. The molluscan host of *C. myocerca* is *Scrobicularia tenuis* which belongs to the same family as *Cumingia.*

Summary

The life cycle of *Lepocreadium setiferoides* (Miller and Northup) has been experimentally traced. The rediae and cercariae develop in the snail, *Nassa obsoleta;* the cercariae encyst in annelids of the genus *Spio* and the turbellarian, *Procerodes warreni;* and the adult worm develops in the intestine of sand dabs and flounders. The structures of the various stages in the life cycle are described.

The anatomical features and most of the life cycle of *Cercaria cumingiae* n. sp. are described. The sporocysts and cercariae develop in the digestive gland of the bilvalve, *Cumingia tellinoides.* The cercariae encyst in the siphons, foot, and other organs of this mollusk. The adult worm probably develops in some fish. From the nature of the excretory bladder, the flame cell pattern, and the location of the genital primordium, it is believed that this trematode belongs to the family *Allocreadiidae.*

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